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(54) FILLER FOR OPTICAL ISOMER SEPARATION FOR LIQUID CHROMATOGRAPHY

(57)Abstract:  
PROBLEM TO BE SOLVED: To provide a filler and a column for liquid chromatography, giving superior optical isomer separation relative to an object compound.  
SOLUTION: In this filler for optical isomer separation for liquid chromatography, made of a polysaccharide derivative carrying filler as a main component and a column in which the filler is filled, a TS coefficient defined by the following formula (I) is in the range of 0.25 to 1.0, TS coefficient=[Vc-t(t(TS)-t(blank)) x FR]/(t(TS)-t(blank)) x ER (I) [In the formula, abbreviations mean Vc (cm3): a column volume, FR (ml/min.): a flow velocity, t (TS) (min.): an elution time of Tetrakis(trimethylsilyl) silane (=TS), and t(blank)(min.): elution time for TS in the state with the column not connected].

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## CLAIMS

[Claim (e)]

[Claim 1] The bulking agent for optical-isomer separation for liquid chromatography characterized by the range of TS multiplier defined by the bottom type (I) obtained using the column for optical-isomer separation for liquid chromatography which filled up column tubing with the bulking agent concerned by the slurry filling-up method in the bulking agent for optical-isomer separation for liquid chromatography which uses a polysaccharide derivative support bulking agent as a main component being 0.25 to 1.0.  
TS multiplier =  $[V_c - t(TS) - t(blank)] \times FR$  /  $[t(TS) - t(blank)] \times FR$  (I)  
[— the elution time amount of TS in the condition of not connecting the elution time amount t (blank) (min.); column of  $V_c$  (cm<sup>3</sup>); column volume FR (ml/min.); rate-of-flow t(TS) (min.); Tetrakis (trimethylsilyl) silane (= TS) is shown among a formula.]

[Claim 2] The bulking agent for optical-isomer separation according to claim 1 for liquid chromatography whose polysaccharide derivative is the ester derivative or carbamate derivative of a cellulose or an amylose.

[Claim 3] The bulking agent for optical-isomer separation according to claim 1 for liquid chromatography which is a bulking agent with which the column for analysis used for the purpose of optical-purity measurement is presented.

[Claim 4] The bulking agent for optical-isomer separation according to claim 1 for liquid chromatography which is a bulking agent with which the column for preparative isolation used for the aliquot of the single column method aiming at optically-active-substance acquisition is presented.

[Claim 5] The bulking agent for optical-isomer separation according to claim 1 for liquid chromatography which is a bulking agent with which the column for preparative isolation of continuous system liquid chromatography is presented.

[Claim 6] The column for optical-isomer separation for liquid chromatography characterized by the range of TS multiplier defined by the formula (I) according to claim 1 being 0.25 to 1.0 in the column for optical-isomer separation for liquid chromatography which uses a polysaccharide derivative support bulking agent as a main component.

[Claim 7] The column for optical-isomer separation according to claim 6 for liquid chromatography whose polysaccharide derivative is the ester derivative or carbamate derivative of a cellulose or an amylose.

[Claim 8] The column for optical-isomer separation according to claim 6 for liquid chromatography which is a column for analysis used for the purpose of optical-purity measurement.

[Claim 9] The column for optical-isomer separation according to claim 6 for liquid chromatography which is a column for preparative isolation used for the aliquot of the single column method aiming at optically-active-substance acquisition.

[Claim 10] The column for optical-isomer separation according to claim 6 for liquid chromatography which is a column for preparative isolation of continuous system liquid chromatography.

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## DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the optical-isomer analytical skill which has a high separation factor and carries out optical resolution of the broad chiral compound in analysis of drugs, food, agricultural chemicals, perfume, etc. especially about the bulking agent and column which are used for separation of an optical isomer, especially separation of the optical isomer by the liquid chromatography method.

[0002]

[Description of the Prior Art] Although physical properties, such as chemical property, for example, the boiling point, the melting point, and solubility, are completely the same, many physical and optical isomers with which a difference is seen by bioactive exist in an organic compound. The living body consists of protein which consists of L-amino acid, and the difference of recognition of the organic compound by the high order dissymmetry space which these protein builds discovers him as a bioactive difference. The difference in the pharmacological activity by the ease of carrying out of association with a specific acceptor in the living body is often studied, and drug effect and the case where a remarkable difference is seen in respect of toxicity are well known for the case of drugs between optical isomers. For this reason, the Ministry of Health and Welfare has indicated Drug Approval and Licensing Procedures when the drug concerned is racemic modification, it is "desirable" to examine absorption, distribution, a metabolic turnover, and an elimination moving state about each isomer.

[0003] As stated previously, research of the physical and the technique of analyzing the optical isomer of a broad class with a simple and sufficient precision since physical properties, such as chemical property, for example, the boiling point, the melting point, and solubility, are completely the same, and it cannot analyze with the usual separation means of an optical isomer was done energetically. And the optical-resolution method by high performance liquid chromatography (HPLC), especially the optical-resolution approach by the chiral column for HPLC progressed as tools of analysis which meet these demands. With the chiral column said here, the chiral stationary phase which made the dissymmetry discernment agent itself or the dissymmetry discernment agent support on suitable support is used. For example, the ovomucoid (JP.63-307829.A) which are optical-active polymethacrylic acid triphenylmethyl (refer to JP.57-150432.A), a cellulose or an amylose derivative (Y. Okamoto, M.Kawashima and K.Hatada, J.Am.Chem.Soc., 106, 5337, 1984), and protein is developed.

[0004] It is known that the column for optical resolution which made the cellulose or the amylose derivative support on silica gel also in the chiral stationary phase for HPLC of these many has high dissymmetry discernment ability to a very broad compound, and examination of the optically-active-substance liquid chromatography method aliquot in the industrial scale which combined such chiral stationary phases for HPLC and a false moving-bed method is further advanced in recent years (12 Phram Tech Japan, vol. 43 (1996)).

[0005] In order to raise the basis of such backgrounds, and chromatography preparative isolation productivity, the chiral stationary phase which gives good separation of a piece is increasingly called for from the purpose compound, and the device which acquires high chromatography

effectiveness boils many things, and it is put.

[0006]

[Means for Solving the Problem] this invention persons reached this invention, as a result of inquiring wholeheartedly about the bulking agent for optical-isomer separation which made the polysaccharide derivative the dissymmetry discernment agent.

[0007] That is, this invention offers the bulking agent for optical-isomer separation for liquid chromatography characterized by for the range of TS multiplier defined by the bottom type (I) obtained using the column for optical-isomer separation for liquid chromatography which filled up column tubing with the bulking agent concerned by the slurry filling-up method to be 0.25 to 1.0, and the column which filled up the list with this in the bulking agent for optical-isomer separation for liquid chromatography which uses a polysaccharide derivative support bulking agent as a main component.

[0008]

TS multiplier =  $[V_c - \{t(TS) - t(\text{blank})\}] \times \text{FR} / [t(TS) - t(\text{blank})] \times \text{FR} (I)$

[— the elution time amount of TS in the condition of not connecting the elution time amount t (blank) (min.); column of  $V_c$  (cm<sup>3</sup>); column volume FR (ml/min.); rate-of-flow t(TS) (min.); Tetrakis (trimethylsilyl) silane (= TS) is shown among a formula.]

[0009]

[Embodiment of the Invention] Hereafter, the gestalt of operation of this invention is explained to a detail.

[0010] The polysaccharide derivative used for this invention is obtained by making a polysaccharide and the compound which has the hydroxyl group and the functional group which can react react.

[0011] Although either a synthetic polysaccharide, a natural polysaccharide and a natural product conversion polysaccharide may not be asked as a polysaccharide used for this invention, but what kind of thing may be used as long as it is optical activity, the high thing of the desirable regularity of a joint format is desirable. if it illustrates — beta-1, 4-glucan (cellulose), and alpha-1, 4-glucan (an amylose —) An amylopectin, alpha-1, 6-glucan (dextran), beta-1, 6-glucan (BUSUTSURAN), Beta-1, 3-glucan (for example, curdlan, sizofiran, etc.), alpha-1, 3-glucan, beta-1, 2-glucan (Crown Gall polysaccharide), beta-1, 4-galactan, beta-1, 4-mannan, alpha-1, 6-mannan, beta-1, 2-cell tongue (inulin), It is beta-2, 6-cell tongue (levan), beta-1, 4-xylan, beta-1, 3-xylan, beta-1, 4-chitosan, alpha-1, 4-N-acetyl chitosan (chitin), a pullulan, agarose, an alginic acid, etc., and the starch containing an amylose is also contained. In these, the cellulose which can obtain the polysaccharide of a high grade easily, an amylose, beta-1, 4-xylan, beta-1, 4-chitosan, a chitin, beta-1, 4-mannan, an inulin, curdlan, etc. are desirable, and especially a cellulose and an amylose are desirable.

[0012] Although the number averages degree of polymerization (the pyranose contained in 1 molecule or the number of averages of a furanose ring) of these polysaccharides is ten or more preferably five or more and especially an upper limit does not have it, it is desirable that it is 1000 or less in respect of the ease of handling.

[0013] Moreover, if it is an isocyanic acid derivative, a carboxylic acid, ester, acid halide, an acid-amide compound, a halogenated compound, an aldehyde, alcohol, or the compound that has a leaving group in addition to this as a compound which has a hydroxyl group and the functional group which can react, what kind of thing may be used and such aliphatic series, an alicyclic group, aromatic series, and a hetero aromatic compound can be used. Especially a desirable thing is the carbamate derivative or ester derivative of a polysaccharide which has 0.1 or more the urethane bonds or ester bonds per 1 glucose unit as a polysaccharide derivative used for this invention.

[0014] With the polysaccharide derivative support bulking agent of this invention, the polysaccharide derivative made to apply on support is used for a raw material. The chemical bond between support and the applied polysaccharide derivative, the chemical bond of the polysaccharide derivatives on support. By the radical reaction using a reaction, a radical initiator, etc. which are caused by the electromagnetic wave exposure of radiation irradiation, such as a chemical bond which used the third component, an optical exposure to the polysaccharide

derivative on support, and a gamma ray, microwave, etc. etc. The bulking agent to which firmer immobilization was given by making the further chemical bond form is also contained. Not using the polysaccharide derivative made to apply on support furthermore, the polysaccharide derivative support bulking agent produced by the approach of carrying out the chemical bond of a polysaccharide or a polysaccharide derivative, and the support, such as silica gel, directly is also contained. Moreover, the bulking agent for optical-isomer separation which uses a polysaccharide derivative support bulking agent as a main component means mixture with the bulking agent which are not objects for optical-isomer separation, such as an above-mentioned polysaccharide derivative support bulking agent, a bulking agent for optical-isomer separation of other type, or silica gel by which octadecyl surface treatment was carried out, for example.

[0015] As support used for this invention, porosity organic support or porosity inorganic support is mentioned, and it is porosity inorganic support preferably. A thing suitable as porosity organic support is a high polymer which consists of polystyrene, polyacrylamide, polyacrylate, etc., and things suitable as porosity inorganic support are a silica, an alumina, a magnesite, glass, a kaolin, titanium oxide, a silicate, hydroxyapatite, etc. Especially desirable support is silica gel. 0.1 micrometers - 10nm of particle size of silica gel is 1 micrometer - 300 micrometers preferably, and 10A - 100 micrometers of average apertures are 50A - 50000A preferably. In order to eliminate the effect of a residual silanol, as for a front face, it is desirable to perform surface treatment, but it is satisfactory even if surface treatment is not performed at all.

[0016] The elution time amount of the tetrakis (trimethylsilyl) silane (it is called Following TS) in the condition of not connecting with the condition of having connected the column to liquid chromatograph equipment in TS multiplier calculation in this invention is measured, and TS multiplier defined by the above-mentioned formula (1) is computed using the acquired elution time amount. Although the analysis apparatus used in the case of this measurement has RI detector, a UV detector, etc. which can check the elution of TS as a detector which is HPLC equipment and is used, it is desirable to detect on the wavelength of 210nm using a UV detector especially.

[0017] As analysis conditions, it carries out on normal phase conditions, i.e., the mobile phase conditions which use a hydrophobic solvent as a main component. Specifically, it is the mobile phase of the presentation ratio of n-hexane / 2-propanol = 9 / 1 (v/v). Moreover, analysis temperature is a room temperature (25 degrees C), and as for the rate of flow, 1/1, especially 4,15 of the quadrant of column volume  $V_c$  (cm<sup>3</sup>) - 9 minutes, i.e.,  $[V_{cx}(1/4,15)]$  ml/min., are desirable. It is still more desirable the amount of volume of 1/300 - 1/600 of column volume and for the amount of placing of TS to drive in especially TS solution made to dissolve TS in a mobile phase by 5.0mg /ml] concentration the amount of volume of 1/415, i.e.,  $[V_{cx}(1/415)]$  ml.

[0018] In this invention, if it is required for the range of TS multiplier computed as mentioned above to be 0.25 to 1.0 and it separates from this range, good separability ability cannot be obtained.

[0019] Although it is common to use for the optical-isomer separation by a chromatography method and membrane separation, such as a gas chromatography, liquid chromatography, supercritical chromatography, thin-layer chromatography, and capillary electrophoresis, as for the bulking agent of this invention, applying to especially a liquid chromatography method is desirable.

[0020] Furthermore, the bulking agent of this invention is preferably used for the column for analysis of the liquid chromatography used mainly for the purpose of optical-purity measurement, the column for preparative isolation of the liquid chromatography of the single column method aiming at several mg - several kg optically-active-substance acquisition, the column for preparative isolation of the continuous system liquid chromatography represented by the simulated moving bed method, etc.

[0021] Hereafter, although an example explains this invention to a detail, this invention is not limited to these examples.

[0022] Example 1 TS multiplier = amylose of 0.527 Aminopropyl silanizing (APS processing) was performed by making the production approach \*\* silica gel surface treatment porosity silica gel

(particle size of 20 micrometers, 1300A of average pore size) of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation react with 3-aminopropyl triethoxysilane by the well-known approach. The silica gel with which carbamoyl surface treatment was performed was obtained at reacting the obtained APS processing silica gel with 3 and 5-dimethylphenyl isocyanate.

[0023] \*\* amylose bottom of synthetic nitrogen-gas-atmosphere mind of tris (3, 5-dimethylphenyl carbamate), and amylose 10.0g -- desiccation pyridine 360ml -- inside and under 3 and 5-dimethylphenyl isocyanate 82.2g (3Eq) and pyridine reflux temperature, it poured into methanol 8.0L, after performing heating stirring for 80 hours. The depositing solid-state was separated with the glass filter, and performed the vacuum drying (80 degrees C, 5 hours) after several washing with the methanol. Consequently, 35.3g (95%) of white solid-states which were yellowish a little was obtained.

[0024] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 10g was dissolved in 100ml of ethyl acetate, and the moiety of this polymer dope was applied to homogeneity at silica gel 40g of \*\*. It is the target amylose by performing reduced pressure drying for 20 minutes on condition that 50 degrees C and 120Torr, remaining ethyl acetate further, and performing reduced pressure drying for 20 minutes for a moiety on the same conditions (50 degrees C, 120Torr) as the point after homogeneity spreading similarly after spreading. The tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained.

[0025] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0026] Example 2 TS multiplier = amylose of 0.926 Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0027] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced.

[0028] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 52.5g was dissolved in 489ml of ethyl acetate, and 1/4 amount of this polymer dope was applied to homogeneity at silica gel 97.5g of \*\*. Reduced pressure drying for 15 minutes was performed for ethyl acetate on condition that 50 degrees C and 120Torr after spreading, and reduced pressure drying for 15 minutes was similarly performed for 1/4 amount to the pan on the same conditions (50 degrees C, 120Torr) as the point after homogeneity spreading. It is the target amylose by carrying out reduced pressure drying of the 1/4 amount for 45 minutes on these conditions (50 degrees C, 120Torr) after homogeneity spreading succeedingly, remaining at the end and carrying out reduced pressure drying of the 1/4 amount for 45 minutes on these conditions (50 degrees C, 120Torr) after homogeneity spreading. The tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained.

[0029] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0030] Example 3 TS multiplier = amylose of 0.286 Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0031] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced.

[0032] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 1.25g was dissolved in 12.5ml of ethyl acetate, and the whole quantity of this polymer dope was applied to homogeneity at silica gel 11.25g of \*\*. It is the target amylose by performing reduced pressure drying for 15 minutes for ethyl acetate on condition that 50 degrees C and 120Torr after spreading. The tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained.

[0033] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0034] Example 4TS multiplier = amylose of 0.696 Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0035] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced. [0036] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 50.25g was dissolved in 437.2ml of ethyl acetate, and 1/3 amount of this polymer dope was applied to homogeneity at silica gel 117.25g of \*\*. Reduced pressure drying for 15 minutes was performed for ethyl acetate on condition that 50 degrees C and 120Torr after spreading. It is the target amylose by carrying out 1/3 amount of a polymer dope after spreading, carrying out reduced pressure drying of the ethyl acetate for 15 minutes on these conditions similarly succeeding, remaining, performing the homogeneity spreading back for 1/3 amount, and performing reduced pressure distilling off for ethyl acetate by the reduced pressure drying for 25 minutes. The tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained.

[0037] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0038] Example 5TS multiplier = amylose of 0.379 Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0039] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced. [0040] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 27.0g was dissolved in 270ml of ethyl acetate, and 1/2 amount of this polymer dope was applied to homogeneity at silica gel 153.0g of \*\*. Reduced pressure drying for 15 minutes was performed for ethyl acetate on condition that 50 degrees C and 120Torr after spreading. They are the reduced pressure drying for 15 minutes, and the target amylose at these conditions after spreading and about ethyl acetate similarly in 1/2 amount of a polymer dope succeeding. The tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained.

[0041] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0042] Example of comparison 1TS multiplier = 1.050 amyloses Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking

agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0043] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced. [0044] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 2.5g was dissolved in 18.75ml of ethyl acetate, and 1/4 amount of this polymer dope was applied to homogeneity at silica gel 3.75g of \*\*. Reduced pressure drying for 15 minutes was performed for ethyl acetate on condition that 50 degrees C and 120Torr after spreading. Succeedingly, the spreading back was performed for 1/4 amount of a polymer dope, and reduced pressure drying for 30 minutes was similarly performed for ethyl acetate on these conditions. Furthermore, it is the target amylose by performing the spreading back for 1/4 amount of a polymer dope, performing reduced pressure drying for 30 minutes for ethyl acetate on these conditions similarly, remaining.

[0045] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

[0046] Example of comparison 2TS multiplier = amylose of 0.240 Carbamoyl surface treatment was performed to porosity silica gel (particle size of 20 micrometers, 1300A of average pore size) as well as \*\* of the production approach \*\* silica gel surface treatment example 1 of the bulking agent for tris (3, 5-dimethylphenyl carbamate) support optical-isomer separation.

[0047] \*\* Amylose By the same technique as \*\* of the synthetic example 1 of tris (3, 5-dimethylphenyl carbamate), it is an amylose. Tris (3, 5-dimethylphenyl carbamate) was produced. [0048] \*\* Amylose Amylose obtained by the support above-mentioned \*\* to the silica gel of tris (3, 5-dimethylphenyl carbamate) Tris (3, 5-dimethylphenyl carbamate) 125.0g was dissolved in 1250ml of ethyl acetate, and the whole quantity of this polymer dope was applied to homogeneity at the 2375.0 g silica gel of \*\*. The target amylose tris (3, 5-dimethylphenyl carbamate) support mold bulking agent was obtained after spreading by performing reduced pressure drying for 10.5 minutes for ethyl acetate on condition that 50 degrees C and 120Torr.

[0049] \*\* Amylose produced by packed column production \*\* for HPLC from a production bulking agent Using the separating medium which supported tris (3, 5-dimethylphenyl carbamate) on silica gel as a bulking agent, the column made from stainless steel with a die length [ of 25cm ] and a bore of 0.46cm was filled up with the slurry filling-up method, and the separation column for optical isomers was produced.

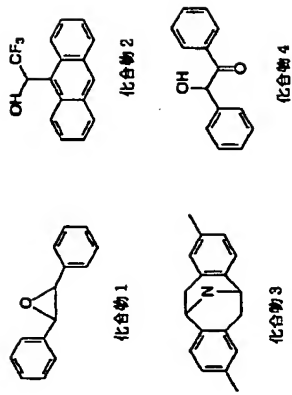
[0050] Amylose produced in the application examples 1-5 and the examples 1-2 of a comparison Using the column for optical-isomer separation for HPLC filled up with the bulking agent which supported tris (3, 5-dimethylphenyl carbamate) on silica gel, the elution time amount [t (TS) (min)] of TS was measured by the liquid chromatography method of the following conditions, and TS multiplier was computed by the following formula. A result is shown in Table 1.

<Analysis condition of liquid chromatography> mobile phase: n-hexane / 2-propanol = 9 / 1 (v/v) rate-of-flow: -- 1.0 ml/min. temperature: -- 25-degree-C detection: -- 210nm placing TS concentration: -- 5.0mg (mobile phase)/ml

The amount of TS placing : 10microlTS multiplier formula >Vc.0.23x3.14x25=4.15cm3. FR:10ml/min. t(blank): 0.16min.TS multiplier = The column for optical-isomer separation for HPLC produced in examples 1-5 and the examples 1-2 of a comparison to [4.15-t(TS)-0.16] x1.0] / [t(TS)-0.16] x1.0 pan is used. Optical resolution of the compounds 1-4 expressed with the following formula which is racemic modification was performed, and the degree-of-separation Rs value which is the index which shows extent of separation of each optically active substance was computed by the following type. The result is also shown in Table 1.

[0051]

[Formula 1]



[0052]  $Rs=2(t1-t2)/(W1+W2)$   
(Here, t1 and t2 show W1, and the elution time amount of each optical isomer and W2 show the peak width of an optical-isomer peak.)

[Table 1]

HPLC用 カラム	tR (分)	TS係数	分離度 (Rs)			
			化合物1	化合物2	化合物3	化合物4
1	2.07	0.627	4.91	1.89	1.98	1.77
2	2.16	0.926	3.40	1.06	1.44	1.12
3	3.14	0.286	3.66	1.03	1.21	1.60
4	2.42	0.696	3.96	1.21	1.63	1.34
5	2.94	0.379	5.49	1.38	1.90	1.91
比 例	1	2.03	1.050	2.17	0.63	1.01
	2	3.26	0.240	2.28	0.59	0.65
						1.21

[0054] Moreover, the relation between TS multiplier of a bulking agent and Rs value of a compound 1 was shown in drawing 1, and the relation between TS multiplier of a bulking agent and Rs value of compounds 2-4 was shown in drawing 2.

[0055] From the above result, the bulking agent which has TS multiplier in the range of 0.25 to 1.0 is understood that the separability ability of an optical isomer is good.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing.1] It is drawing showing the relation between TS multiplier of a bulking agent, and Rs value of a compound 1.

[Drawing.2] It is drawing showing the relation between TS multiplier of a bulking agent, and Rs value of compounds 2-4.

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[Translation done.]

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